NOTES ON THE BIONOMICS OF STEGOMYIA CALOPUS, Meigen, IN BRAZIL

PART II

BY

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THE ABSENCE OF STEGOMYIA CALOPUS LARVAE FROM NATURAL WATERS

From December, 1920, to February, 1922, a fairly extensive search was made in the town of Manáos, its native suburbs, and the sparsely inhabited forest surrounding it, for the breeding-places of various mosquitoes. During this examination *Stegomyia calopus* larvae were never found, except in domestic waters in the immediate vicinity of a human habitation. Young (1921), writing from Manáos, has drawn attention to the same point; Howard, Dyar and Knab (1912) state: 'The larvae are found practically exclusively in artificial receptacles about human habitations. It may be said that the larvae of *calopus* are never found in swamps, in pools or in temporary puddles, even when these are in close proximity to houses.'

The three experiments that follow were devised to test whether the absence of larvae from such waters was due to the disinclination of females to oviposit in them, or to the inability of the larvae to develop when placed there.

Some small stagnant pools situated on the outskirts of the town, about fifty yards from six native houses and about the same distance from the tram line, were selected for the experiments; water from pools of this description will be referred to in the text as 'natural water,' in contra-distinction to the term 'domestic water' as applied to water in rain barrels, water troughs, etc.

Experiment I. A varying number (4 to II) of S. calopus females were confined in two breeding cages, and in each cage were placed six large watch glasses containing water from various sources, both natural and domestic; the position of these glasses was constantly varied to prevent any undue influence of light or shade. Males were introduced and the supply kept constant. Sugar solution was supplied for the males; the females were fed on human blood, a feed being usually offered every other day. The resultant eggs were removed and counted every twenty-four hours. The experiment was continued for five weeks, with the results recorded below.

	Nature of water supplied	Total number of eggs deposited	Percentage deposited in each type of water
I	Distilled water	146	9.6
2	Barrel water in which wild Stegomyia were freely breeding	88	5.8
3	Water from a small pool on the outskirts of the town ; this pool harboured <i>Culex</i> and dragon-fly larvae	149	9*8
4	Deep pool near (3); contained <i>Culex</i> but no dragon- fly larvae	309	20'4
5	Same as (4), but algae added	728	48.1
6	Small pool, same source as (3) and (4). Culex and dragon-fly larvae, but no vegetation	92	6.0

TABLE I.

CONCLUSION. Stegomyia in captivity will oviposit as readily in natural as domestic waters. This conclusion agrees with that of Bacot (1916), and of Fielding (1919), but both these authors used domestic water throughout their experiments, and to this added various organic substances.

Experiment II. To ascertain whether *Stegomyia* ova and larvae can develop in natural waters, when these have been cleared of inhabitants inimical to the life of the larvae. Eight jars were used; six of these contained 400 c.c. of water and two 800 c.c., all the waters being carefully strained through fine wire gauze, before the introduction of the larvae.

		I	

No. of Jar	Nature of the water used	No. of Ova added	No. of larvae which hatched	No. of Imagoes	Percentage of Ova which completed cycle	Average time taken to complete cycle
1	Tap water, plus 2 grs. of rice	24	23	8	33	days 28
2	Water from a barrel in which Stegomyia were freely breeding	24	24	10	41	24
3	Smalı pool natural water which contained dragon-fly and water- beetle larvae	30	21	3	IO	22
+	Small pool natural water which contained no insect life	30	26	25	83	21
5	Tap water plus 2 grs. of rice	30	22	3	IO	37
6	Small pool natural water which contained dragon-fly and <i>Culex</i> larvae	30	25	23	70	25
7	800 c.c. water as in Jar 2	30	28	11	36	17
8	800 c.c. water as in Jar 3, plus well- washed duck weed	30	2	13	43	10

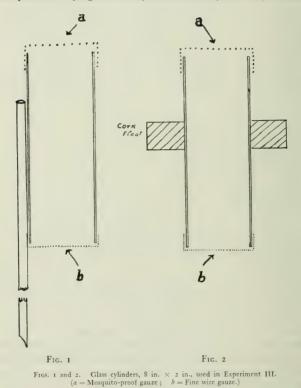
CONCLUSION. *Stegomyia* ova hatch and the larvae develop freely in natural waters after these have been freed from insects inimical to their development.

Experiment III. To ascertain whether *Stegomyia* larvae can develop in pools of natural water when (1) unprotected from their insect enemies, (2) protected from their insect enemies.

A small pool such as is described under Experiment I was selected. A careful netting of the pool showed the following inhabitants :---dragon-fly larvae, tadpoles, a few water bugs (*Zaitha* sp.), a small water beetle (previously shown to be harmless to mosquito larvae). No culicidae larvae were found, though the neighbouring pools showed large numbers, mostly *Culex fatigans*.

Into this pool were introduced 900 dried eggs and a few fresh eggs of *Stegomia calopus* (average fertility of dried eggs was found to be about 40 per cent.), also 300 larvae of *Stegomyia* and 200 larvae of *C. faligans*, the larvae being on an average 48 hours old. Two glass cylinders,

arranged as shown in fig. **I**, were fixed to pointed sticks and these sunk into the mud at the bottom of the pool, about three inches of the cylinder being left projecting above the surface of the water. Into one tube were introduced 50 dried eggs, and 24 fresh eggs of *Stegomyia*. Into the other were placed 20 *C. fatigans* larvae (not more than 24 hours old) During



the course of the next seven days the pool was regularly visited, but, unfortunately, a week's heavy rain interfered with the observations and the experiment was brought to a close on the eighth day by the rising water in the pool completely submerging the tubes. The following results were obtained. In spite of very careful searching no *Stegomyia* larvae were found in the pool during the eight days it was kept under observation, the first search being made twenty-four hours after the introduction of the ova and larvae; *C. fatigans* were present during the whole experiment, although on two occasions no larvae could be found; this was probably due to the muddy condition of the water. Of the larvae in the guarded cylinders, both lots appeared to be doing well, three *Stegomyia* imagoes emerging during the eight days; many of the *C. fatigans* larvae reached the fourth stage, but none pupated.

A few months later the experiment was repeated with the following modifications :—600 *calopus* and 255 *faligans* larvae at all stages of development were added to the pool, and in the cylinders were placed respectively, 26 *calopus* ova (average fertility of a sample found to be 90 per cent.) and 26 *faligans* ova (average fertility 100 per cent). In lieu of fixing the cylinders to sticks they were attached to cork floats (fig. 2) and allowed to float clear in the pool. The results obtained were precisely similar to those in the previous experiment, except that no imagoes were obtained though both tubes contained apparently healthy larvae. As before, *calopus* larvae disappeared after the first twenty-four hours, while *faligans* persisted. The observations were brought to a close on the seventh day by the drying of the pool.

SUMMARY. Stegomyia calopus ova and larvae, introduced into a natural pool infested with insect enemies, disappeared after the first twenty-four hours, whereas Culex fatigans larvae, added under the same conditions, persisted for at least eight days. S. calopus ova placed in the same pool, but under conditions protecting them from insect enemies, developed and produced imagoes.

ORDER OF HATCHING OF MALES AND FEMALES

Rees (1901) states: 'When mosquitoes are bred in captivity the males as a rule hatch out first, and in greater numbers than the females.' Nuttall and Shipley (1901) comment on this statement as follows: — 'We have found no similar statement elsewhere, and the observations we have made do not tend to confirm his observation. The proportion of males to females has always appeared to us to be fairly equal, and we have counted the sexes on several occasions.' Bacot's (1916) observations in West Africa would appear to confirm Rees. Writing of *Stegomyia* he says :

'... the early males being usually a day quicker in their development than the females.'

The following note deals only with the order of hatching, Young (1922) having already dealt with the proportion of males to females. As it appeared possible that the food supply might influence the sexes differently, an attempt was made to breed the larvae on different food supplies, other factors being kept as nearly as possible equal. To do this, mixed batches of eggs were sunk and the larvae within twelve hours of hatching transferred to jars containing one of the following two food supplies :-(I) Minimum food supply, viz., tap water to which was added 0.015 per cent. polished rice and 0.5 grm. well-washed duck-weed to each 300 c.c.'s. water. (2) Maximum food supply, viz., stagnant river water filtered through fine wire gauze, to which was added 0.018 per cent. Peptone (Fairchild) and 0.5 grm. well-washed duck-weed to every 300 c.c. of water. Each larva was allowed 30 c.c. of the prepared water, this amount being regulated every day; thus to start with, ten larvae were placed in 300 c.c. of the food supply ; if two died within twenty-four hours then the amount of water was reduced to 240 c.c. and so on. The results are shown in the following tables :----

TABLE III.

Maximum	food suppl	y. Num	ber of l	larvae = 270.
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Day of emergence	5	6	7	8	9	10	11	12	13	14	15	Total
Males	0	3	33	58	20	25	3	0	0	0	0	142
Females		I	10	35	24	24	3	I	o	0	0	98

TABLE IV.

M	inimum	food	suppl	ly.]	N	um	ber	of	larvae	-	305	
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Day of emergence	8	9	10	TI	12	13	14	15	16	17	18	19	20	21	22	23	2.4	25	26	27	28	Tot
Males	0	5	17	- 19	9	10	11	6	9	9	5	2	0	0	0	0	I	I	0	I	0	105
Females	. 0	0	I	8	IO	8	18	II	14	10	7	3	4	3	0	1	I	0	2	0	0	101

SUMMARY. In a mixed batch of ova, hatched and a'lowed to develop under food conditions which were either (I) favourable, or (2) adverse to growth, it was found that a much greater number of males than females reached maturity during the first few days of the emergences. This preponderance of males was greater than could be explained by the higher proportion of males to females (I42 to 98, and I05 to I0I) as observed in the completed experiments.

OVULATION

Experiment I. Results of Diets other than Blood.

Goeldi (1905), in Brazil, after numerous experiments with fruit, sugar, honey, etc., came to the conclusion that blood was necessary for the production of eggs by *Stegomyia calopus*. Fielding (1919), working in Australia with the same species of mosquito, obtained fertile eggs on three occasions on which peptone and sugar was given as a food. Ken (1917), in India, fed *Stegomyia scutellaris* on sugar, milk and sugar, peptone and sugar, with positive results.

S. calopus females were kept under observation for a period of over twelve months, at least twenty being always present in the cages. During this time the ordinary food supplied was sugar and water, and on two occasions a mixture of sugar and peptone was given for several days; the results were similar to those already published by Young (1922), no instance of egg laying being recorded.

Several authors record mosquitoes feeding on plants; thus Theobald (1901), states: 'I have frequently seen Culicidae settled on Compositae sucking the juices of the flowers, both males and females,' and Giles (1902), states: 'When mosquitoes are unable or unwilling to obtain blood they suck the juices of plants.' Knab (1907), quotes other instances. The following two experiments were made to see whether *Stegomyia* would feed on flowering plants, and if so, whether ova would result.

(1) Thirteen females were confined for thirty-eight days in a cage and supplied with water and a variety of flowering plants, representing as nearly as possible all the species growing within a ten-yards' radius of a heavily infected breeding place (a disused water barrel); in all seventeen species of plants were used, and each plant was allowed to remain in the cage for three to four days. In addition to the plants the fruits 'Goiaba' and banana were supplied. Males were always kept present. Both males and females constantly alighted on the flowers, inserted their proboscis in the corolla, and apparently absorbed some fluid. (2) Seven females were observed under the same conditions for twentyone days, but the following additional fruits were used : Melon, 'Mammão,' Mango, Orange, 'Periba,' 'Caju,' the results being the same as in the first experiment.

A trial was then made of the following native fruits : (3) Three females fed for thirteen days on Mango, 'Mammão,' Melon, Orange, Banana. (4) Five females fed for thirteen days on Mango, Orange, Banana, 'Periba,' 'Mammão.' No eggs were laid in either of the latter experiments.

SUMMARY. Female *Stegomyia* were offered and fed readily on sugar, sugar and peptone, flowering plants, and various fruits. No eggs were laid after feeding on any of the above substances.

Experiment II. Results of feeding on animals and birds with special reference to Bats and Wall Geckos.

Durham (1902), MacGregor (1915), Bacot (1916), Theobald (1916), and Fielding (1919), record *Stegomyia* feeding on Dog, Goat, Rat, Bandicoot, Agouti, and Guinea Pig; the results of the author's experiments of feeding *S. calopus* on various animals and birds are recorded in the following table.

No.	Animal or bird used and method of feeding adopted.	Whether seen to feed	Ova laid	Whether fertile
1	A small finch confined in mosquito cage day and night on eight occasions	No	0	-
2	A rock-dove confined in mosquito cage day and night on four occasions	No	o	_
3	Young parrots; the tube containing the mosquitoes was applied to the host's body	Yes	+	-1
4	Domestic chickens; method of feeding as above	Yes	+	+
5	A young otter; method of feeding as above. (Only a little blood absorbed, partly due to restlessness of animal)	Yes	0	_
6	Lesser Ant Bear; method of feeding as above. (Only half- hearted attempts made to pierce the skin)	No	o	_
7	Monkey; method of feeding as above	Yes	+	+
8	Cotia; method of feeding as above	Yes	+	+
9	Young Iguana (Urocentron azureum) confined in mosquito cage for some days and nights	No	0	-
10	Young Wall Gecko, placed loose in cage and also enclosed in tight fitting gauze bag; several trials day and night	No	0	_
11	Bats (Molossus obscurus) left loose in cage; several experi- ments tried both day and night	Yes	.+	+

TABLE V.

The above list requires no comment, except for the last two animals named. In all houses observed in Amazonas, whether deserted or occupied, two animals were constantly found present, viz., the gecko and various species of bats. Special attention was, therefore, devoted to seeing if *Stegomyia* would feed on these in the absence of human blood. The experiments with the gecko were frequently repeated, using both young and adult specimens. At first it was allowed loose in a cage of hungry mosquitoes, none of which attempted to bite. The gecko destroyed numbers of the mosquitoes, so in subsequent observations it was enclosed in a tight-fitting gauze bag and placed on the bottom of the cage. Though mosquitoes were often seen to alight on the gauze and probe it tentatively, they were never seen to draw blood, nor were any females gorged in the morning if the gecko and bag were allowed to remain in the cage over night.

The only record noted of mosquitoes feeding on bats is that of Durham (1902) at Pará, who observed a *Stegomyia calopus* female feed on 'a small bat (Phyllostoma).' The following species of bats were found to be common in or around houses in Manáos : *Saccopteryx bilineata*, Tenum., *Hemiderma perspicillatum*, L., *Vampyrops zarhinus*, H. All., *Molossus rufus*, Geoff., *Uroderma bilobatum*, Pet., *Molossus obscurus*, Geoff. Of these *Molossus obscurus* appeared to be the commonest in houses, and was used in the following three feeding tests. (I) Eleven offered a feed and nine fed; (2) three offered, two fed; (3) six offered, six fed. Not only did a far higher percentage of those given the opportunity feed on bats than on other animals, but they appeared to attack their host with a far greater voracity than they were observed to exhibit towards any other creature except man. They usually settled on and pierced the wing membranes, and as soon as they were flicked away returned to the attack, the complete feed being thus performed in a series of interrupted bites.

CONCLUSION. Stegomyia calopus feeds with great readiness and voracity on bats; it appears likely that these serve as important food reservoirs in deserted houses, or sparsely inhabited districts. It will also feed, but with less eagerness, on certain other animals and birds. All attempts to induce Stegomyia to bite the common wall gecko failed.

Experiment III. Results of feeding on washed red cells, serum, and citrated blood.

Otto and Neumann (1905), in Brazil obtained fertile eggs by feeding *Stegomyia* on blood and salt solution. Bacot (1916), in West Africa, on

two occasions obtained single eggs by feeding the mosquitoes, on one occasion on honey and blood, and on the other on syrup and blood, one of these eggs proving fertile. Marchoux and Simond (1906), at Rio imprisoned eight female *Stegomyia* and fed them as follows: Two on fresh human

No.	No. of female Stegomyiae	No. of days observed	Food : and how offered	No. ot Ova laid
Ŧ	6	15	Washed sheep's cells in normal saline. 0.5 c.c. in a watch- glass	0
2	5	17	Washed sheep's cells in normal saline. 0'5 c.c. in a watch- glass	23
3	7	19	Sheep's serum. 0.5 c.c. in a watch-glass	0
4	7	24	Washed human red cells in normal saline. 0.5 c.c. in a watch-glass	0
4a	3	9	Washed human red cells in normal saline in tubes; same mosquitoes as in (4)	0
5	6	26	Human serum. 0.5 c.c. in a watch-glass	0
5a	3	5	Human serum, in tubes ; same mosquitoes as in (5)	0
6	6	29	Whole human blood in saline. 0.5 c.c. in a watch-glass	0
6a	3	8	Whole human blood in tubes with normal saline : same mosquitoes as in (6)	0
7	3	IO	Cotton ball soaked in whole human blood plus normal saline, suspended in cage	0
8	3	16	Cotton ball soaked in washed human red cells in normal saline, suspended in cage	0
9	3	13	Cotton ball soaked in human serum suspended in cage	0
10	2	14	Cotton ball soaked in washed human red cells plus normal saline, suspended in cage	0
11	3	8	Cotton ball soaked in whole human blood plus normal saline. suspended in cage	30*
12	3	12	Cotton ball soaked in human serum, suspended in cage	0

T				[.]

* 29 hatched

serum, two on red cells separated by centrifuging, two on blood-clot, and two, which were used as a control, on themselves. The last two laid eggs, the remainder did not. In the following experiment males were always present in the cages, and water supplied for the reception of eggs. The food was renewed every two or three days, in some instances every day. In Table VI (p. 434), under the column 'Food,' the expression 'in tubes' refers to the method used by Rodhain and others (1912) for feeding tsetse flies : small segments of glass tubing were covered at one end with the thin skin of a bat, filled with the food to be tested and suspended in the cage. This proved to be a very satisfactory method of feeding ; the proboscis of the mosquito could be clearly seen piercing through the membrane into the fluid and sometimes the absorption of food could be observed ; but neither by this nor by any other method, were the mosquitoes induced to feed to repletion as they do on the living animal.

SUMMARY. In a series of experiments in which fifty-four females were offered as food either serum, washed red cells, or whole blood (the two latter being diluted with normal saline), it was found that the mosquitoes absorbed any of the fluids offered, but that oviposition only resulted in the case of whole blood.

BITING OF CADAVERS

Rosenau and others (1904) quote two instances of *Stegomyia* feeding on native corpses, respectively half an hour and twelve hours after death. Christy (1900) observed mosquitoes (Anophelines) feeding on a white man's corpse in Nigeria three and a half hours after death.

The mosquitoes mentioned in the following notes were first tried on the living subject, and, if found willing to feed, were given the opportunity of biting the cadaver. All experiments were conducted in dav-light.

(1) Native Brazilian, dead three hours. Three females used ; all three bit, only one filled with blood.

(2) Native Brazilian, dead six and a half hours. Three females used ; two bit, none absorbed blood.

(3) Native Brazilian, dead seven hours. One female used, which bit and absorbed blood.

(4) Native Brazilian, dead eighteen hours. Three females used ; all three bit, none absorbed blood.

CONCLUSIONS. *Stegomyia calopus* females will bite corpses as long as eighteen hours after death, and will draw blood as long as six hours after death.

NATURAL ENEMIES OF STEGOMYIA CALOPUS

Young (1921) has dealt with the natural enemies of the larval *Stegomyia* and the value of dragon-flies in the destruction of the adults in Amazonas. The following note deals with the enemies encountered in dwelling-houses. Bacot (1916) considers toads, lizards, spiders, ants, scorpions, and possibly young Mantidae to be destroyers of *Stegomyia*. Marchoux and Simond (1906) at Rio found a jumping spider of the genus *Salticus* was a foe of *Stegomyia*, and came to the conclusion that it was probably of some practical importance. Neither of these authors publish any exact data.

In the experiments that follow an attempt was made to estimate the value of the animal as a mosquito destroyer, first by allowing it to feed on mosquitoes only, and then under conditions which gave it a choice between mosquitoes and other food likely to occur in its natural environment. Bats, wall geckos, and a species of jumping spider, were found to be common in all houses in Manáos. With regard to bats, twelve were killed in houses in Manáos; none of these showed any mosquito remains in the gut, nor were any scales found in the faeces.

Experiment I. A small wall gecko, about four and a half inches long was confined in a mosquito cage; the intervals between the feeds varied from a few minutes to several days.

		S. calo	pus			C. fati	gans		Other insects, Flies, Grasshoppers, etc.				
No.	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	4	After 12 hours	2.1	At Start	After 4 hours	12	2.4	
I	25	6	I	1	0	-	-	_	0	-	-	-	
2	7			5	I	-	-	0	29	-	-	several	
3	6			I	5		—	I	9	-	-	0	
4	2			I	I	-	-	0	15	-	-	7	
5	8	-	-	3	2	-	—	0	14	_	-	3	
6	2	_	1	-	2	-	2	-	16	-	7	-	
7	3	-	-	0	4	-	-	0	22	-	-	13	

TABLE VII

Experiment II. A jumping spider (Family Salticidae, genus Akela) which was found to be very common in all houses in Manáos, was confined either in a mosquito cage or in a small glass aquarium. As before, the intervals between feeds varied greatly.

No.	S. calopus				C. fatigans				Other Insects, Flies, Grasshoppers, etc.			
	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours	At Start	After 4 hours	After 12 hours	After 24 hours
I	3	0	-	-	0	-	-	-	0	_		_
2	16		4	0	0	_	-	_	o	_	-	
3	5	0	_	_	0		-	-	0		-	
+	15		-	_	0	_	_	_	0	-	-	_
5	11	3	_	0	O				0		-	
6	17	I	0	-	3	_	0	_	0			_
7	8	3	_	0	0	_		-	5	2	_	0
8	0	-	-	_	IO	2	-	-	0	_	-	_
9	0	-	-		45		_	21	6		-	3

TABLE VIII

CONCLUSIONS. Both the wall gecko and a species of jumping spider (genus Ahela), were found readily to destroy *S. calopus* and *C. fatigans*, and owing to their wide distribution are probably of some importance in limiting the numbers of mosquitoes occurring in human habitations. Their usefulness is, however, limited by the fact that other insects besides mosquitoes form part of their diet. An examination of twelve bats revealed no mosquito remains in the intestines.

STEGOMYIA CALOPUS BREEDING NATURALLY ON BOARD A SHIP VOYAGING FROM MANÁOS TO LIVERPOOL

Marchoux and Simond (1906) record placing 20 male and 20 female *S. calopus* in breeding tubes when leaving Rio in February, and on arrival at France, in May, twelve females and nine males were still alive. The insects during the voyage were fed on human blood.

The following observations were made in the month of February. Two days after leaving Pará a considerable number of Stegomvia were still to be found in various parts of the ship, and as their numbers appeared to be increasing, the whole ship was searched for possible breeding places. Eventually several hundred larvae were found in each of two large glass vases in the passengers' smoke room, both the jars containing cut-palm leaves which had been taken on board at Manáos. The larvae when found were at least forty-eight hours old, and as the steward stated that the vases had been cleaned and refilled about seven days previously, the ova must have been deposited shortly after leaving Manáos. When Havre was reached, sixteen days after leaving Pará, one jar contained many living imagoes, pupae, and larvae, the other, imagoes only. Liverpool was reached twenty days after leaving Pará, and twenty-four hours after the vessel had reached port, numerous living Stegomvia adults, both males and females, were present in both jars; in addition one of the jars contained a number of active pupae. The morning (2.0 a.m.) deck temperature varied between 81° F. at Pará and 53° F. in Liverpool.

CONCLUSION. *Stegomyia calopus* is capable of completing its cycle on board a ship travelling between Manáos and Liverpool, and the resultant adults can remain alive until arrival in Liverpool.

NATIVE MOSQUITO REPELLENTS

The natives who inhabit the forests surrounding Manáos are reported to employ two vegetable substances, 'Tocum' and 'Urucu,' to repel the attacks of biting insects. No opportunity occurred of testing the value of 'Tocum.' In regard to 'Urucu,' which Da Matta (1912) states is *Bixa orellana*, the seeds from the shrub of this name are crushed and added to nut oil, the resultant mixture being smeared over the exposed parts of the body. Da Matta (1912) agrees with the native belief that insects will not bite persons so protected. A sample was obtained and rubbed over one arm, both arms being then thrust into a large mosquito cage, containing hungry *Stegomyia* females. The number of subsequent bites on each arm were as follows :---

Number of	bites	on arm	treated wi	ith 🗇	Urucu	۰.	•• •		13	
Number of	bites	on unti	reated arm				••		15	
ONCLUSION.	The	native	preparatio	n kn	own a	as '	Urucu	' is	of	n

value as a repellent of S. calopus.

C

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